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THE EFFECT OF 353' L-TRIIODOTHYROPROPIONIC ACID ON PLASMA LIPIDS

by

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INTRODUCTION:

Written records of man's interest in the thyroid gland date from the second century A.D., when Galen described this organ and attributed to it the function of providing a lubricating fluid to the larynx and its cartilages to facilitate speech. This theory was accepted until as recently as the 17th century by such prominent scientists as Bartholin and Malpighi. In the 18th century and the early part of the 19th, the theory was proposed that the thyroid functioned "as a diverticulum in order to avert from the brain a part of the blood, which urged with too great force by various causes might disorder or destroy the functions of that important organ." Experimental research on the thyroid, however, was not perforemed to any appreciable extent until the latter half of the 19th century; although from the fact that sheep's thyroid was administered for the treatment of cretinism in China as early as the 6th century A.D., one can probably infer that physicians at that time were aware of some relationship between growth and the thyroid. From thyroidectomies on monkeys and carnivora, Horsley in 1885 supported the generalization proposed by Felix Simon two years previously that myxedema, cretinism, and operative cachexia strumipriva were all due to thyroid deficiency.[78] And, in 1891 Murray restored myxedematous patients to normal health with the administration of thyroid extract.[53]

Shortly after the turn of the 20th century, in 1914, Kendall isolated from the thyroid gland an active principle in crystalline form which he named "thyroxin".[34] In 1926 the constitution of this crystalline substance was established by Harington to be that of an amino acid related to tyrosine, and this substance was subsequently synthesized.[26, 28-30]

Since the isolation of thyroxine and diiodotyrosine from extracts of thyroid tissue and their synthesis, in the past forty odd years, much research has been carried out on the influence of the thyroid secretions on the processes of growth and development; on metabolic rate; on carbohydrate, fat, and protein metabolism; and on the activity of various enzymic systems. Furthermore, numerous compounds other than thyroxine itself have been found to possess some of the activities of extracts of the thyroid gland. The literature on these various topics is so voluminous that no attempt to review them will be made in the present paper. Nevertheless, in spite of all these advances, one encounters in the 17th edition of the Textbook of Physiology edited by John F. Fulton in 1956 the following statement by Jane Russell: "The mechanism of action of the thyroid hormone is unknown."[75]

Another momentous step in the understanding of the physiology and biochemistry of the thyroid was made when Gross and Pitt-Rivers identified the compound 1-353' triiodothyronine in the plasma of patients after the administration of radioactive iodine.[23] The activities of this substance have subsequently been extensively investigated. It has been found to be three to four times as potent as l-thyroxine in preventing the formation of goitre in thiouracil-fed rats.[24] L-triiodothyronine given to a patient with classical myxedema was found to act much more rapidly than thyroxine in raising the Basal Metabolic Rate (BMR), but the total response to thyroxine was about threefold that of triiodothyronine. The same investigators studying the half-life of exogenously administered I^{131}-labeled l-triiodothyronine and l-thyroxine, discovered that of the former to be $2\frac{1}{2}$ days and of the latter to be 7 to 12 days.[54]

In 1952, Harington and Pitt-Rivers synthesized the acetic analogue of l-thyroxine.[27] Subsequently tetraiodothyroacetic acid (TETRAC) has been identified in extracts of kidney and triiodothyroacetic acid (TRIAC) in extracts of liver from mice which had received an intraperitoneal injection of I^{131} eighteen to forty-eight hours prior to sacrifice.[17] With intravenous injections of TRIAC into rats in doses as minute as 1/16 ug and in intevals as frequent as one-fourth of a minute, Thibault has found that this compound, in contrast to thyroxine and triiodothyronine, exerts an immediate effect in increasing the oxygen consumption of the entire animal. Although the maximum effect was observed to be only about 60% of that of thyroxine, but because this effect was detected immediately, Thibault postulated that thyroid hormones acted at the peripheral tissue level in the form of the acetic acid derivatives.[74] Barker, however, was unable to find any evidence for the immediate action of TRIAC from his studies on oxygen consumption of tissue slices removed from animals following injections of this analogue.[74] The question of the active form or forms of thyroid hormones at the level of peripheral tissues, therefore, still remains unsettled.

In addition to thyroxine, triiodothyronine, and TRIAC about forty other thyroid analogues have been synthesized and studied in terms of their effectiveness in mammals in descreasing the uptake of radioactive iodine by thyroid gland, in preventing the development of goitre in animals fed with thiouracil, in enhancing the rate of basal metabolism, and in promoting the rate of metamorphosis of tadpoles. 353' l-triiodothyropropionic acid (TRIPROP) an interesting compound in this group, was found to be 300 times more potent than l-thyroxine in accelerating

metamorphosis of the tadpole, though only 3/4 as efficacious in reducing the uptake of I^{131} by thyroid gland of the rat, and ½ as active in preventing the formation of goitre in rats fed with thiouracil.[48, 49, 65] However, at the time the present study was undertaken, very little work had been done concerning the actions of this substance in human beings.

Although the symptoms and signs of typical cases of thyroid dysfunction are well known, and diagnosis in such instances can be made without hesitation, there are many cases with atypical features enabling one to make only an equivocal diagnosis. For many years the rate of basal metabolism has been used as an indication of thyroid activity, a rate of below - 20% being accepted as a criterion of hypothyroidism. However, it has been pointed out that the basal metabolic rate (BMR) has not proved to be an infallible guide in the diagnosis of thyroid dysfunction, as it may be low without the syndrome of sensitivity to cold, puffiness of skin, coarse hair, and mental and physical retardation. On the other hand, Gildea, Man and Peters showed that with pre-treatment levels of serum cholesterol instead of BMR, one could predict with greater certainty the response of these patients to treatment with desiccated thyroid. They also found that serum cholesterol levels in hypothyroid patients were very sensitive to the administration of thyroid. Although in these patients, the amounts of total serum fatty acids tended to vary with the cholesterol levels, the relationship between the two was not strictly linear. Moreover, total fatty acid levels in hypothyroid people also seemed to be less responsive to thyroid therapy.[18] However, in contrast to the usefulness of serum cholesterol concentrations in predicting the response of hypothyroid states to treatment, they appeared to be of no assistance in predicting the response of questionable hyperthyroidism to thyroidectomy. In a study

of a series of forty-three patients exhibiting most or some of the classical symptoms of hyperthyroidism, Man, Gildea, and Peters observed that two of the patients with initially low lipid values showed little rise in serum cholesterol and other lipids although exhibited a definite reduction in BMR after thyroidectomy. In 11 of 26 patients of this same series studied for more than six months following thyroidectomy, the serum lipids tended to level off to a plateau after a postoperative rise in spite of the fact that a concomitant elevation in BMR was not seen.[41]

Much evidence has been accumulated with regard to the relationship between thyroid function and lipid metabolism. Only a few examples will be cited. As early as 1917, Epstein and Lande noted that in diseases of the thyroid, serum cholesterol tended to vary inversely with the basal metabolism.[15] Marx, Gustin, and Levi discovered that thyroxine stimulated and thyroidectomy depressed the incorporation of deuterium into cholesterol in the liver, intestine, kidneys, and spleen but not in the lungs of rats.[44] Rats made hyperthyroid by feeding powdered thyroid were found to excrete at least twice as much cholesterol into the bile as the euthyroid controls, but rats treated with thiouracil excreted only about half the amount eliminated by control animals.[59] And, as mentioned previously, hypothyroid patients after treatment with desiccated thyroid showed a reduction in levels of serum cholesterol concomitant with improvement in the clinical state.[18] Serum cholesterol levels, however, do not seem to be correlated closely with the body's immediate caloric or metabolic requirements.[35, 40] On the other hand, the importance of serum free fatty acid (FFA) in the transport

of metabolic fuel has only very recently come to the foreground.

In a group of 82 subjects from an unselected population, Dole observed that the fasting levels of FFA ranged from 0.315 to 1.21 meq/l and that there was a reduction of serum FFA of about 65% one and half hours after the administration of glucose and of about 50% two and half hours after the subcutaneous injection of insulin in a dose of 0.1 unit per kg of body weight.[12] Similar observations were made independently by Gordon and Cherkes.[20] Subsequently Bierman, Dole, and Roberts showed that although there was not much difference between the average fasting FFA levels in nondiabetic, obses individuals and diabetics without ketosis, diabetics in ketosis exhibited a definite elevation in serum FFA.[7] Gordon, Cherkes, and Gates from studying arterio-venous differences in human subjects have discovered that FFA concentrations in venous blood draining mainly subcutaneous tissues, such as that in the greater saphenous vein, are definitely higher than those found in arterial blood, which were again higher than those found in blood samples obtained from the coronary sinus.[21] More recently Carlson and Pernow have produced evidence to indicate an increased extraction of FFA from plasma by muscular tissues during moderate exercise in a fasting state.[10] One can thus infer from these various experiments that serum concentrarions of FFA vary inversely with the body's need for or ability to utilize carbohydrate. Consequently, FFA levels are intimately related to the body's metabolic requirements, and the FFA portion of serum lipids assumes an important role in the transport of fuel from the depots to the active tissues. Because of the significant position of FFA in the total metabolism, an inkling of the influence, if any, of

353' l-triiodothyropropionic acid on lipid metabolism can perhaps be ascertained from a study of the effect that this particular thyroid analogue exerts on the nonesterified portion of the serum fatty acids.

In investigations of whole-animal metabolism, one parameter that has often been used by physiologists is the respiratory quotient. The molecular structures of fats and carbohydrates are such that the combustion of a unit quantity of fats requires more molecules of oxygen than the combustion of an equal quantity of carbohydrates. With this fact as the basis for calculations, the percentage of fat or carbohydrate utilized in a certain period for the support of the organism's metabolism can be estimated by measuring the total amount of carbon dioxide expired and oxygen used, and tables with this kind of information are available in most physiology textbooks.

That euthyroid and hypothyroid people respond differently to exogenously administered thyroid substance has been demonstrated by Winkler, Riggs, and Man. In hypothyroid patients, desiccated thyroid in a dose sufficient to cause clinical improvement, but in all cases as small as 1 to 3 grains per day, was effective in elevating concentrations of serum precipitable iodine (SPI) from below 2.5 ug% to an average of 4.8 ug%. The increase in SPI per grain of desiccated thyroid was also remarkably constant, the mean being 2.0ug% per grain with a standard deviation of ± 0.5 ug%. On the other hand, in euthyroid subjects, frankly abnormal levels of SPI, i.e., greater than 8ug%, were not consistently maintained until the dose of desiccated thyroid reached or exceeded 10 grains per day.[57, 72] Furthermore, nonmyxedematous subjects can tolerate as much as 6 grains of desiccated thyroid for months

without any effect on pulse rate or BMR. Euthyroid subjects respond to intravenous thyroxine, but they require much larger doses than myxedematous subjects for the same rise in BMR.[71] This difference in response has been postulated to be on the basis of decreased inactivation of thyroid hormones in myxedematous individuals.[57, 72]

The present porject, then, is designed to study the influences, if any, of 353' l-triiodothyropropionic acid on plasma lipids and transport and utilization of fats in euthyroid human subjects with the hope that the results might contribute toward some understanding of the actions of substances possessing thyroid-like activities.

METHODS:

The effects of 353' l-triiodothyropropionic acid, (TRIPROP), on the levels of serum lipids in human beings were studied through both an acute and a more prolonged experiment. All experimental subjects were students at the Yale University School of Medicine with ages ranging between 23 and 26. At the time of the study, they were all enjoying good health. None of them had any previous history of hypothyroidism, hyperthyroidism, diabetes mellitus or essential hyperlipemia. Each of the two experiments will now be described separately.

1. The acute experiment. Three subjects were studied: V.M., male; J.W., female; and P.L., male. After an overnight fast lasting for twelve to thirteen hours, blood samples were drawn early in the morning, 7:00 or 8:00 a.m. or the arbitrary zero hour, of the control day for analyses of FFA, total fatty acids, lipid phosphorus, and cholesterol, and gas samples were collected. Then at 1, 2, $5\frac{1}{2}$, $8\frac{1}{2}$, 12, and 24 hours after the collection of the first sample, blood samples were obtained for the analysis of FFA. In addition, sufficient blood was collected for the study of lipid phosphorus, total fatty acids, and cholesterol at $8\frac{1}{2}$ and 24 hours after the first sample. Gas samples were obtained at $5\frac{1}{2}$, $8\frac{1}{2}$ and 24 hours after the first sample. Throughout these 24 hours after the overnight fast, subjects did not ingest any food but were allowed as much water as they liked.

A rest period of one week elapsed after the control day, and on the experimental day subjects were asked to carry out the same activities as on the control day. Every procedure on the experimental day was performed in a manner identical to that on the control day with the

following exceptions: (a) Subjects received TRIPROP[*] at a dose of 0.3 mg/kg of body weight[**] immediately after the collection of the first blood and gas specimens at 0 hour, and (b) blood samples were obtained for the determination of butanol extractable iodine, (BEI), at 0, 1, $5\frac{1}{2}$, $8\frac{1}{2}$, 12 and 24 hours from subject V.M. and at 0 and $5\frac{1}{2}$ hours from subject P.L.

2. The prolonged experiment. Four subjects were studied: W.R., male; M.W., female; E.L., female; and R.M., male. Blood and gas samples were collected at 8:00 a.m. In addition, subjects took their own pulse rate every morning upon arising; blood pressure was determined every day after the venepuncture; and daily weights were obtained. Subjects were allowed their regular diets and were asked to perform similar activities every day. The control study took place on three consecutive days, and the experimental study was carried out through a period of six consecutive days following the control period. During the experimental period, every procedure was the same as on the control day with the exception that each subject took TRIPROP at a dosage of 0.3 mg/kg body weight every morning with his breakfast shortly after the venepuncture and gas collection for the respective day. However, the values of the various determinations on the first day of the experimental period should actually be comparable to those of the control period, as subjects did not start

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[*] TRIPROP donated by Warner Chilcott.

[**] This particular dosage was chosen mainly because some of Dr. M. J. Albrink's patients were receiving as much as 12-18 mg/d without any toxic effect.

taking TRIPROP until after the first venepuncture and gas collection. Determinations of plasma lipids and of respiratory quotients were performed daily. BEI studies, however, were done only on the 1st, 3rd and 5th days on subject M.W. and on the 1st, 4th and 6th days on subject E.L.

All specimens used for the analyses of plasma lipids were venous blood samples drawn into heparinized syringes. From the syringe blood was rapidly transferred into round bottom pyrex tubes and centrifuged at room temperature at the rate of about 1500 rpm for a period of 10 minutes. For FFA determination, a proper aliquot of plasma was withdrawn into a calibrated Van Slyke pipette, transferred to a round bottom tube, and to it a measured amount of extraction mixture was added. The entire extraction procedure was carried out within thirty minutes after the venepuncture. For determinations of lipid phosphorus, cholesterol, and total fatty acids, plasma samples were stored in the refrigerator, and extraction procedures were carried out within one week after the venepuncture. Venous blood specimens for the study of BEI were drawn into heparinized iodine-free syringes prepared by Dr. E. B. Man's laboratory.

For the determination of respiratory quotient, gas samples were collected prior to the venepuncture in the following manner: each subject, after having had his nose artificially obstructed, breathed through a rubber mouth piece connected to a low pressure double-valve apparatus the outlet of which was in turn joined to a Douglas bag and the inlet of which communicated freely with room air. After the subject had been resting physically for at least 20 minutes, expired gas was collected for a period varying from 3½ to 5 minutes, the exact duration

being determined with a stop-watch. Gas samples were transferred into air-tight tonometers within 20 minutes after collection and the concentrations of carbon dioxide and oxygen were analyzed with a Scholander gas analyzer.[62]

All FFA determinations were performed according to the method of Dole[12] with the modification that instead of thymol blue, Nile blue prepared according to Gordon[20] was used as the indicator. A palmitic acid standard extracted with the same procedures as the blood samples was run with each group of FFA determinations. Titrations for FFA levels of subjects of the acute experiment were performed with a Rehberg microburette and of the chronic experiment, with a Gilmont microburette.

Extraction of plasma lipids of subjects of the acute experiment were performed according to the method of Man and Gildea.[39] Then 5 or 8 ml aliquot of the alchol-ether filtrate was withdrawn for the determination of lipid phosphorus by a modification of the method of Fiske and Subbarow.[64] For the determination of total fatty acids and total cholesterol, 40 ml aliquot of the alcohol-ether filtrate was saponified with alcoholic potassium hydroxide, according to Man and Gildea.[39] The remainder of the analysis was performed with the following modifications. After evaporation of this alcohol-ether filtrate, the potassium salts of the serum fatty acids were acidified with concentrated hydrochloric acid. The saponified free fatty acids were then suspended in about 12 ml of distilled water, and to this suspension was delivered exactly 20 ml of n-hexane measured with a volumetric pipette. This mixture in a stoppered flask was shaken vigorously and then transferred

into a 50 ml centrifuge tube. The centrifuge tube was fitted with a ground glass stopper and the mixture centrifuged for 20 minutes at 2000 rpm. Then 1 or 2 ml aliquot of the upper hexane phase was withdrawn for titration of total fatty acids with 0.02N NaOH, and 3 ml aliquot of the same phase was obtained for determination of cholesterol by the method of Abell.[1]

Determinations of concentrations of total fatty acids, lipid phosphorus and cholesterol of subjects of the chronic experiment were performed with the extraction and titration of Albrink[2] and the cholesterol method of Abell.

All determinations on plasma lipid fractions were carried out in duplicates, and as many analyses of aliquots of gas from one single specimen as were necessary to give two values with a difference of not greater than 0.03% were run with the Scholander apparatus.

Lipid phosphorus determinations were carried out by Dr. Albrink's laboratory, and BEI determinations were performed by Dr. Man's laboratory. Analyses of all gas samples and of the remaining plasma lipid fractions were done by the author.

The amount of total cholesterol in mg/100 ml was converted to the amount of cholesterol fatty acids in meq/l with the following formula:

$$\text{Chol fatty acid in meq/l} = \frac{(\text{chol in mg/100 ml})\ (0.72)\ (10)}{386}$$

The amount of phospholipid fatty acids in meq/l was calculated from the amount of lipid phosphorus in mg/100 ml with the following formula:

$$\text{P lipid fatty acids meq/l} = \frac{(10)(\text{Lipid P mg/100 ml})\ [(0.80)(2)+0.2]}{31}$$

These formulae are based on the assumptions that 72% of the serum cholesterol exists in the esterified form, that 80% of the serum phospholipids contains two fatty acid molecules per mole of phosphorus (e.g. lecithin and cephalin), and that the remaining 20% of the serum phospholipids contains only one fatty acid molecule per mole of phosphorus (e.g. sphingomyelin). The amount of triglyceride fatty acids represents the difference between the total fatty acids and cholesterol fatty acids, phospholipid fatty acids, and FFA. Lipid phosphorus was converted into phospholipids by multiplying by a factor of 25; the cholesterol-phospholipid ratio was thus expressed as cholesterol in mg per 100 ml/(25) (lipid P in mg/100 ml).[8]

Calculations of respiratory quotients required certain assumptions, namely, that the composition of room air was essentially constant from day to day, that the percentage of oxygen in room air was 20.93% and that of nitrogen 79.04%, and that the concentration of carbon dioxide in room air was so minute that the volume of this gas expired per unit time could be considered to be equivalent to the volume of carbon dioxide produced by the body's metabolism. The volume of gas collected in the Douglas bag was measured through a Precision Wet Test flowmeter under room temperature and pressure. The volume of gas expired per minute was then determined and corrected to standard temperature and pressure. The respiratory quotient was calculated with the following formulae:

1. $$O_2 \text{ inhaled/min} = \frac{(\%\ O_2 \text{ in room air})\ (\%\ N_2 \text{ in expired air})\ (\text{corrected minute vol. of exp. air})}{\%\ N_2 \text{ in room air}}$$

2. $$O_2 \text{ expired/min} = (\%\ O_2 \text{ in exp. air})\ (\text{corrected minute vol. of exp. air})$$

3. O_2 utilized/min = O_2 inhaled/min - O_2 exhaled/min.

4. CO_2 produced/min = (% CO_2 in expired air) (corrected min. vol. exp. air)

5. Respiratory quotient = $\frac{CO_2 \text{ produced/min.}}{O_2 \text{ used/min}}$ 73

It should be noted that the respiratory quotient thus obtained takes into consideration the combustion of carbohydrates, fats, and proteins. However, the amount of protein metabolized remains quite constant in an individual whose diet is about the same from day to day and in an individual undergoing a short term of starvation during which his liver glycogen and adipose tissues serve as the sources of metabolic fuel.

RESULTS:

The acute experiment:

The one study on the rate of absorption during fasting state and duration of effect of exogenous TRIPROP as measured by levels of serum BEI in subject V.M. revealed that the highest BEI level was obtained at $5\frac{1}{2}$ hours after the ingestion of this compound (Table 8). However, since no blood sample was obtained prior to $5\frac{1}{2}$ hours, one cannot be certain whether the peak level had not been reached any earlier. Two other similar studies, one while the subject abstained from food for 24 hours and the other while the subject was on a regular diet, showed that the peak BEI level in the former was reached two hours after administration of the drug, and in the latter, one hour after.[78] In all three cases BEI levels returned to approximately the original, i.e., pretreatment, values within four days after the administration of the drug.

However, levels of BEI as high as 19 ug/100 ml of serum did not appear to have any definite or consistent effect on the concentrations of serum FFA, cholesterol, lipid phosphorus, triglycerides, and on the respiratory quotient (Tables 1-6, Figs 1, 3). The levels of serum FFA in all subjects on both the control and experimental days reached a peak at the 12th hour. However, only one subject, V.M., showed a concentration higher on the experimental than on the control day, the level on the former being 1.23 meq/l, while that on the latter being 0.81 meq/l. Subject P.L., on the other hand, actually showed the reverse pattern, namely, the peak level on the control day being 0.457 meq/l higher than that on the experimental day. In subject J.W., however,

the curves of FFA levels on the control and experimental days can almost be superimposed on each other.

With regard to the levels of plasma cholesterol, both P.L. and J.W. exhibited an elevation as the end of the fasting, control day drew near; P.L. showed an increase of 13.6% and J.W., 8.6%. On the other hand, in both these subjects, concentrations of plasma cholesterol tended to fall as the experimental day progressed. The reduction, however, was small, being about 9.8% in P.L. and only 3.7% in J.W. In subject V.M. not much difference can be detected in the fluctuations on both the control and experimental days.

The levels of lipid phosphorus also showed a variable pattern. In subjects P.L. and J.W., the lipid phosphorus, like the cholesterol, exhibited a rising trend on the control day and a falling trend on the experimental day. On the control day, in subject P.L., the level at the 24th hour was 17.6% higher than that at the 0 hour; and in subject J.W., the increase was 14.1%. The same parameter was observed to fall by 9.7% in P.L. and by 11.6% in J.W. on the experimental day. The response seen in V.M. was quite different from that observed in the other two subjects. Instead of a tendency to reduction, the concentration actually rose by 16.2% on the experimental day; whereas on the control day, although there were some fluctuations within the first six hours, the level reached at the 24th hour was quite similar to that at the 0 hour.

Tables #1 and #4 represent the levels of plasma total fatty acids and triglyceride fatty acids at various time intervals of the control and experimental days. Here each subject seemed to respond in his own

individual manner, no definite pattern being established.

Respiratory quotients are recorded on table #6. On both control and experimental days, all three subjects had a lower respiratory quotient at the 24th hour than that at the 0 hour, the difference ranging from 0.094 to 0.193. on the control day. However, the degree of change on the experimental day was not significantly different from that seen on the control day, the difference between the 0 hour and the 24th hour ranging from 0.047 to 0.132.

The chronic experiment:

The results of this investigation revealed a few interesting points. In the first place, in subject M.W., the levels of BEI dropped in spite of continued administration of TRIPROP. The highest BEI level, 28.3 ug%, was reached after two daily doses of TRIPROP. After the 4th daily dose, however, the BEI was only 19.9 ug%. The response in subject E.L., on the other hand, showed a continued rise with continued treatment. (Table 20)

The levels of plasma cholesterol showed a consistent and considerable reduction with the administration of TRIPROP. This response was detectable as soon as one day after the first dose, and after 5 daily doses the cholesterol levels varied between 62 and 82% of the initial values. Although the levels of cholesterol varied from day to day during the control period, the fluctuations, the greatest of which was 10%, were insignificant compared to the response seen after the administration of TRIPROP (Fig. 4)

The FFA levels as can be seen from Fig. 2 did not show any

consistent pattern with TRIPROP. As a matter of fact, they exhibited considerable variation within the control period. The total fatty acid levels in three of the four subjects showed a downward trend. However, this most likely was a reflection of the response observed in cholesterol. There is no dramatic or consistent change in cholesterol-phospholipid ratio (Table 15).

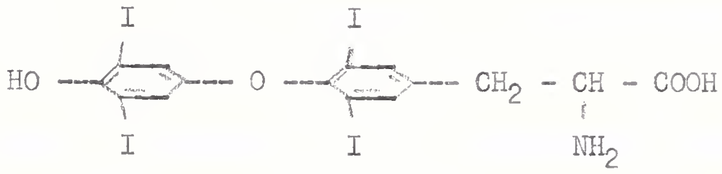
No generalizations can be drawn from the responses of the respiratory quotient. Here each subject appeared to react in a different manner. (Table 14)

The result of measurements of daily blood pressure was remarkable in that no consistent pattern could be detected (Table 19). The morning pulse rate, however, showed an upward trend; although after five daily doses of TRIPROP they were only from 14 to 26 beats/min. more than the initial values (Table 18). All four subjects lost weight on the experimental regimen (Table 17). In two subjects, as much of a reduction as seven pounds in five days was seen. With the exception of the occurrence of mild headaches during the midst of the chronic experiment in two subjects, one of whom had the onset of menstrual period coincident with the onset of the cephalalgia, and transient increase in perspiration in the other of these two subjects, TRIPROP in the dosage given here did not seem to cause any symptoms of nervousness, jitteriness, irritability, increase in appetite, palpitation or diarrhea.

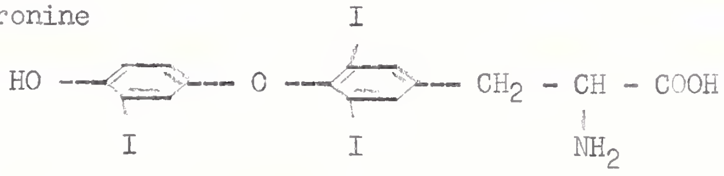
DISCUSSION AND CONCLUSION:

In an attempt to locate the active site or sites of the thyroxine molecule, large scale investigations have been carried out on various metabolic activities of numerous compounds possessing the basic thyronine structure but with varying amino acid and fatty acid side chains as well as different number of iodides at various positions on the thyronine molecule.[48, 49, 65] A clearer concept of this particular approach can perhaps be grasped from examining the following representative formulae:

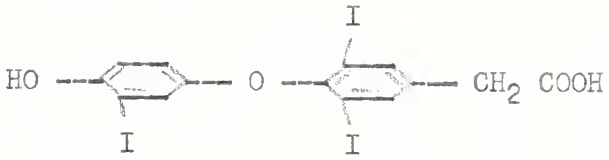
Thyroxine



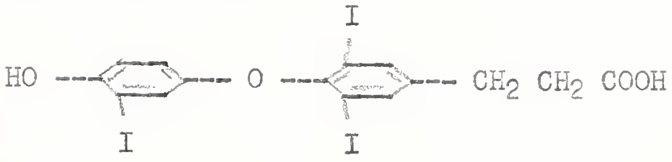
353' triiodothyronine



353' triiodothyroacetic acid



353' triiodothyropropionic acid



It has been found from these studies that within the vertebrates, different classes of animals respond differently to a certain chemical

substance; this phenomenon is, of course, by no means unusual in biology. For example, a study on the rate of metamorphosis of Rana pipiens tadpoles revealed that 353' l-triiodothyropropionic acid (TRIPROP) was 300 times more potent than l-thyroxine in accelerating this process;[49] but another investigation in the rat on the efficacy of various thyroid analogues in suppressing the formation of goitre in animals treated with thiouracil showed that TRIPROP possessed only 25% of the activity of l-thyroxine.[65]

A recent paper by Bauer, Mc Gavack, and Swell reported that the administration of TRIPROP in a dose of 6 mg daily to euthyroid adults caused an average serum cholesterol reduction of 25% after a period of two to four weeks.[5] Turner and Steiner, in 1945, showed that administration of desiccated thyroid in dosages varying from 30 to 240 mg/day to euthyroid subjects caused a sharp decrease in the levels of serum cholesterol.[68] However, from the studies so far available, one cannot arrive at any conclusion in regard to the relative activity of l-thyroxine and TRIPROP in this particular parameter. It is interesting to note, nevertheless, that the subjects of Bauer did not show much variation of the BMR concomitant with the reduction in serum cholesterol, whereas the BMR's of Turner's subjects were definitely elevated, with an average increase of 20%.[5, 68] The present work corroborated the results of Bauer in that significant reductions in serum cholesterol were evident after the administration of TRIPROP. The results in the present work were observed sooner than those in Bauer's; this fact can very likely be attributed to the larger dosage here used. TRIPROP, therefore, seems to exert the same influence on serum cholesterol as

thyroxine and triiodothyronine. Because the present experiment was carried out for only five to six days, one cannot predict from the available data the response of serum cholesterol to more prolonged treatment with TRIPROP at the constant dose of 0.3 mg/kg of body weight. Perhaps it would not be unreasonable to assume that a plateau will eventually be reached after a certain period and that further reduction in serum cholesterol will occur only upon the administration of a larger dose.

Previous workers have concluded that adipose tissues appear to be more than normally active in hyperthyroid states. The low fasting respiratory quotients in patients with exophthalmic goitre[14] and the predisposition to ketosis of hyperthyroid subjects[47] suggest that mobilization and utilization of fats are accelerated. This hypothesis seems certainly to be substantiated by the work of Rich, Bierman, and Schwartz, who have demonstrated that the administration of l-triiodothyronine in a dose of one or two mg led to a definite elevation in the level of serum FFA within 3 to 6 hours.[56] This is significant especially in view of the probable important role played by serum FFA in the transport of fat or metabolic fuel from sites of storage to sites of metabolic activity. The present work, however, does not show any consistent response of the levels of FFA to TRIPROP in as large a dose as 0.3 mg/kg. They seemed to vary up and down in spite of the TRIPROP. It is also difficult to interpret the data on the respiratory quotients (R. Q.), from the present study because not every subject responded in exactly the same manner. In the acute experiment, the R.Q. in all three subjects decreased as the day progressed. However, the percentage reduction in the R.Q. on the experimental day was not really significantly different from that

on the control day. Since the R.Q. reflects the relative proportions of carbohydrate and lipid metabolized by the body, and since the body must rely more and more upon the lipids as the metabolic fuel as the readily available supply of carbohydrate in the body becomes depleted with prolongation of starvation; it is understabdable that as the fasting day progressed, the R.Q.'s indeed became lower. In the chronic experiment, when the subjects were on their regular diets, one subject, M.W., showed a rather constant R.Q. throughout the four days that she was on TRIPROP. Another subject, E.L., showed a distinctly downward trend, the R.Q. on the last day of the experimental period being 0.14 lower than that on the first, pre-TRIPROP, day. Two other subjects, however, exhibited an irregular trend. From these data, one can only conclude that either TRIPROP exerts no effect on fat mobilization and utilization or other more potent factors are present. Because of the lability of the levels of FFA, its rate of turnover being extremely rapid with a half life of only two to three minutes,[31] its sensitive response to changing concentrations of epinephrine and availability of serum glucose; because after 12 to 36 hours of fasting, the livers in different subjects may contain different amounts of glycogen; because of the uncontrollability of the subjects' psychological milieu, hence the secretion of epinephrine, during periods of experimentation; whatever effect TRIPROP might have on the levels of FFA can conceivably be obscured by the actions of these various factors.

In the study by Rich, Bierman and Schwartz alluded to previously, it was shown that administration of 0.15 or 0.2 mg of l-triiodothyronine daily led to a definite, significant elevation in the BMR within the

first week of such treatment.[56] This kind of effect, i.e., the effect of thyroxine and triiodothyronine on the BMR and the increased BMR observed in patients with Graves' disease or toxic nodular goiter, have, of course, been observed by numerous other investigators. As in the case with the respiratory quotient, the present study does not permit any definite conclusion to be drawn regarding the effect of TRIPROP on this particular parameter of metabolic activity. The subject who exhibited the consistently downward trend in her R.Q.'s showed also a consistently upward trend in the BMR. However, since there was so much fluctuation in her BMR during the three days of the control period, it is very difficult to determine the significance of this upward trend during the experimental period. As a matter of fact, one cannot say unequivocally how much significance these nine figures on the BMR actually possess. The subject whose R.Q.'s were relatively constant throughout the experimental period revealed a consistently upward trend in the values of BMR, that on the last day of the experiment being about 20% higher than that on the first day. The other two subjects again showed a random fluctuation, at least superficially.

However, in view of the fluctuations seen in the values of both the R.Q. and BMR in all subjects even during the control period, one must cast some doubt on the accuracy of these determinations. Because of the method of gas collection, any degree of hypoventilation or hyperventilation will give rise to a misrepresentation of the actual amount of carbon dioxide produced and oxygen used by the body's over-all metabolism. Since breathing through a rubber mouth piece with one's nose artificially obstructed is not a natural respiratory avenue

and since the subject's equanimity might have been jeopardized by the previous venepuncture, it is easy for the subjects to hold their breath or to breathe unusually deeply even though they realize the importance of respiring naturally and are willing to cooperate. Furthermore, the resting period before the collection of gas was not rigidly controlled from day to day. The subjects rested from anywhere between 20 to 30 minutes rather than a strict minimum of 30 minutes as has been suggested by other investigators.[4]

Because of the inadequacy and imprecision of the determinations of BMR and R.Q., the varying response of the FFA levels in all seven subjects studied, and lack of information in the present-day scientific literature, no conclusion can be justifiably drawn concerning the influence of TRIPROP on the mobilization and utilization of lipids. Certainly more work of the kind performed in the present study, but perhaps with varying dosages of TRIPROP, concomitant investigation of the levels of serum glucose, and more uniform conditions for the gaseous studies, will be needed before one can arrive at meaningful impressions and conclusions. Even then, only the pharmacologic rather than the physiologic activities of TRIPROP on lipid metabolism can be elucidated.

That TRIPROP exerted some metabolic effects, however, was evident from the fact that all subjects in the chronic experiment showed weight loss ranging from about 2.5 to 7 lbs in the four to five days that they were receiving TRIPROP. Moreover, by the end of the experiment, all subjects had a pulse rate higher than their initial one, the increment ranging from 14 to 22 beats per minute. These effects are similar to those seen in people in various states of hyperthyroidism, be they

pathologically produced as in Graves' disease and toxic nodular goitre, or artifically induced with the treatment of thyroxine or triiodothyronine. It must be mentioned, however, that TRIPROP did not lead to any elevation of systolic pressure. Moreover, with the exception of occurrence of mild headaches in two subjects after the 2nd and 3rd doses of TRIPROP and slight hyperhidrosis in one of these two subjects, the common symptoms of hyperthyroidism were absent. It can at least be said, then, that TRIPROP in some ways duplicate some of the effects of the "natural" thyroid hormones. But whether all these different compounds act at the same loci is still a very unsettled question.

Another interesting aspect of the activity of thyroid hormones and analogues centers upon the questionable likelihood of a dissociation of action. For the past four decades, it has been known that hyperthyroid human subjects exhibit an exaggerated or hypersensitive response to exogenously administered epinephrine, manifesting increase in heart rate, blood pressure, tremor, and palpitations. With carefully regulated intravenous infusions of epinephrine into euthyroid and hyperthyroid subjects as well as patients with normal thyroid function but other hypermetabolic conditions such as Hodgkin's disease, lymphosarcoma, and anxiety neurosis; Murray and Kelly demonstrated that euthyroid individuals could tolerate infusion rates of epinephrine up to 0.2 ug/kg/minute before experiencing symptoms of epinephrine toxicity, whereas hyperthyroid patients could tolerate only fractions of this dose. Although euthyroid subjects responded to the administration of epinephrine with an elevation of pulse rate and blood pressure and an increase in oxygen consumption, the hyperthyroid subjects did so

much more dramatically. The response of serum cholesterol, however, was variable and unpredictable.[50] Other investigators have demonstrated that the oral administration of reserpine to patients suffering from thyrotoxicosis reduces their basal metabolic rate, heart rate, and blood pressure although exerts no measurable influence on either the level of protein-bound iodine or the uptake of radioactive iodine by the thyroid gland.[9] However, the increase in peristaltic activity of the gastrointestinal tract, a prominent feature of hyperthyroid states, is actually reversed by epinephrine.[50] Perhaps some effects of thyroid function are indeed mediated through epinephrine, which for some yet unknown reason appears to be more active in subjects with hyperthyroid states.

With regard to the cholesterol-lowering effect of thyroid hormones, Lerman and Pitt- Rivers reported two cases of myxedema that showed a prompt clinical response and a rapid fall in serum cholesterol but no change in the BMR after treatment with TRIAC.[37] Subsequently similar work was repeated by Trotter, who administered to two myxedematous patients TRIAC, triiodothyronine, and thyroxine in dosages of 2-6 mg q.d., 0.04 - 0.08 mg q.d., and 0.1 - 0.2 mg q.d., respectively but found the responses in the form of reduction of serum cholesterol and elevation of BMR to all three substances to be very much alike. Trotter then studied the reaction of four euthyroid subjects to the administration of gradually increasing doses of TRIAC, up to 4 mg q.d. for a period of about seven months. The result in one of the four cases was unacceptable as the serum cholesterol level was for some unknown reason already falling at the time TRIAC was given, but in the other three subjects

there was a definite fall in the serum cholesterol although the BMR rose only very slightly.[67] In a more recent double-blind study using triiodothyronine, thyroxine, and TRIAC in relative doses of 1:5:50 and lactose as placebo in subjects with nontoxic goitre, Doniach, Hudson, Trotter, and Waddams demonstrated that all three thyroidal substances in such dosage were equally effective in reducing serum cholesterol; but symptoms of clinical hyperthyrodism were observed in none of the nine subjects receiving triiodothyronine, in six of the nine subjects receiving thyroxine, and in three of the thirteen subjects receiving TRIAC.[13] The average maximal change in BMR was +5.45% in those subjects on triiodothyronine, +33.55% in those on thyroxine, and +7.85% in those on TRIAC.[11] Interpretation of the results of these various investigations can only be difficult and uncertain, for it is conceivable that different results would be demonstrated by using different dosages of the drugs. However, one can probably conclude that both TRIAC and triiodothyronine, in a dosage comparable to thyroxine in terms of the effectiveness in reducing serum cholesterol, seem to exhibit less effectivenessthan the latter in causing an elevation of BMR and the appearance of subjective symptoms of nervousness, irritability, perspiration, tiredness and diarrhea.

However, it has been demonstrated by Rich, Bierman, and Schwartz that the intravenous administration of triiodothyronine to euthyroid subjects resulted in a definite rise in oxygen consumption after three to six hours but no change in the level of serum cholesterol within the same period, although the latter was reduced within the first week of continuous oral administration of this compound. There was also a definite

elevation in the plasma FFA concentration with the rise in oxygen consumption.[57] On the other hand, studying the effect of TRIPROP on serum cholesterol and BMR Bauer found that oral administration of 6 mg q.d. for two to four weeks led to a significant reduction in the serum cholesterol but essentially no change in oxygen consumption.[5] The results of the present study are in agreement with those of Bauer's investigation.

Recently the relationship between thyroid function and plasma cholesterol has been investigated from a new and interesting viewpoint, namely, through the interaction of androsterone, which is one of the metabolic products of testosterone. The urinary excretion of androsterone and the conversion of exogenously administered testosterone to androsterone have found to be decreased in hypothyroid states. The production of androsterone relative to etiocholanolone is returned to almost normal degree by therapy with triiodothyronine. Administration of androsterone has been observed to lead to a reduction in levels of plasma cholesterol not only in myxedematous patients, but also in euthyroid patients with hypercholesteremia due to other causes and in subjects with both normal thyroid and cholesterol states. One subject studied exhibited an increase in basal oxygen consumption of about 16% in addition to the reduction in serum cholesterol after receiving androsterone.[32] It is thus interesting to speculate on the "thyromimetic" activity of androsterone on cholesterol. Is the cholesterol-lowering action of thyroid hormones mediated through an increase in the production of androsterone? How does an increase in thyroid function lead to an increased production of androsterone? Through what mechanisms does

androsterone act to effect the observed changes in serum cholesterol? Does androsterone have any other thyroid-like activities such as acceleration of pulse rate, increase in body temperature, enhancement of gastrointestinal motility, and elevation of systolic pressure? Is this another case for the "dissociation of actions" of the thyroid hormones? At present, there are no satisfactory answers to these questions. Further research in these various directions would undoubtedly be fruitful.

From the maze of data presently available, no final word can yet be said in regard to the hypothesis of dissociation of actions of thyroid hormones. The present study has not contributed substantial information to the elucidation of this problem but has shown that the administration of large doses of 353' l-triiodothyropropionic acid can lower plasma cholesterol without causing any of the toxic symptoms of hyperthyroidism. If indeed there be a real dissociation of the various activities of the thyroid hormones, most likely this dissociation would exist between the two types of effects mentioned previously, namely, the actions leading to a lowering of serum cholesterol and those giving rise to symptoms possibly associated with a heightened epinephrine effect, such as jetteriness, nervousness, palpitation, tremor, and elevation of blood pressure.

SUMMARY:

1. The effect of 353' l-triiodothyropropionic acid on plasma lipids was studied by the administration of this substance in a dosage of 0.3 mg/kg of body weight to three subjects in an acute experiment and to four subjects in a chronic experiment; the analyses of plasma BEI, FFA, total fatty acids, triglyceride fatty acids, phospholipid fatty acid; and determinations of respiratory quotient and basal metabolic rate. The acute experiment lasted for 24 hours, during which the subjects fasted. The chronic experiment lasted for a period of five to six days, during which the subjects were on their regular diets. All blood and gas samples were collected early in the morning after an over-night fast.

2. Daily weights and measurements of pulse rate and blood pressure were obtained on subjects of the chronic experiment.

3. No consistent effect of 353' l-triiodothyropropionic acid on plasma FFA, triglyceride fatty acids, phospholipid fatty acids, respiratory quotient, and basal metabolic rate were observed.

4. While on 353' l-triiodothyropropionic acid, subjects of the chronic experiment showed a reduction of plasma cholesterol level of from 18 to 38%, a weight loss of from 2.5 to 7 lbs in five days, an increase in pulse rate of from 14 to 26 beats/minute, but no consistent or significant change in blood pressure.

5. Evidence was presented and discussed in relation to the possibility of a dissociation of the actions of thyroid hormones and analogues.

Table 1 --- Acute Experiment --- Plasma Total Fatty Acids in meq/L

	V.M.		J.W.		P.L.	
	Control	Exp.	Control	Exp.	Control	Exp.
0 hr.	10.22	9.02	9.42	9.46	12.92	11.58
1 hr.	9.86	9.67	--	--	--	--
2 hr.	10.4	--	--	--	--	--
5½ hr.	9.6	--	--	--	--	--
8½ hr.	9.93	--	9.79	10.25	13.29	11.28
12 hr.	9.86	--	--	--	--	--
24 hr.	9.52	--	12.83	10.75	13.08	9.16

Table 2 --- Acute Experiment --- Plasma FFA in meq/L

	V.M.		J.W.		P.L.	
	Control	Exp.	Control	Exp.	Control.	Exp.
0 hr.	.757	.392	.34	.34	.321	.36
1 hr.	.618	.406	.49	.44	.31	.35
2 hr.	.636	.455	.58	.66	--	--
5½ hr.	.641	.569	.82	.72	.541	.59
8½ Hr.	.631	.916	.70	.92	.955	.68
12 hr.	.811	1.23	2.29	2.18	1.3	.84
24 hr.	.686	.791	1.5	1.28	1.11	.77

Table 3 --- Acute Experiment --- Plasma Cholesterol in mg/100 ml

	V.M.		J.W.		P.L.	
	Control	Exp.	Control	Exp.	Control	Exp.
0 hr.	176	158	175	161	199	184
1 hr.	179	155	---	---	---	---
2 hr.	187	164	---	---	---	---
5½ hr.	185	155	---	---	---	---
8½ hr.	180	151	177	162	210	171
12 hr.	178	151	---	---	---	---
24 hr.	180	161	190	155	226	166

Table 4 --- Acute Experiment --- Plasma Triglyceride Fatty Acids in meq/L

	V.M.		J.W.		P.L.	
	Control	Exp.	Control	Exp.	Control	Exp.
0 hr.	2.023	1.738	0.66	1.03	3.61	2.98
1 hr.	1.892	2.374	---	---	---	---
2 hr.	1.494	---	---	---	---	---
5½ hr.	1.449	---	---	---	---	---
8½ hr.	1.649	---	0.86	1.38	3.79	2.97
12 hr.	1.524	---	---	---	---	---
24 hr.	1.184	---	1.53	2.08	---	1.17

Table 5 --- Acute Experiment --- Plasma Lipid Phosphorus in mg/100 ml.

	V.M.		J.W.		P.L.	
	Control	Exp.	Control	Exp.	Control	Exp.
0 hr.	7.17	6.8	8.9	8.78	8.8	8.0
1 hr.	6.92	6.9	---	---	---	---
2 hr.	8.25	---	---	---	---	---
5½ hr.	7.0	---	---	---	---	---
8½ hr.	7.4	---	8.5	8.5	7.6	7.40
12 hr.	7.25	---	---	---	---	---
24 hr.	7.4	---	10.8	7.8	---	6.85

Table 6 --- Acute Experiment --- Respiratory Quotient

	V.M.		J.W.		P.L.	
	Control	Exp.	Control	Exp.	Control	Exp.
0 hr.	.827	.828	.825	.825	.845	.889
1 hr.	---	---	---	---	---	---
2 hr.	---	---	---	---	---	---
5½hr.	.709	.709	.733	.727	.810	.789
8½ hr.	.84	.74	.708	.77	.837	.79
12 hr.	---	---	---	---	---	---
24 hr.	.733	.696	.632	.758	.747	.77

Table 7 --- Acute Experiment --- Cholesterol-Phospholipid Ratio

	V.M.		J.W.		P.L.	
	Control	Exp.	Control	Exp.	Control	Exp.
0 hr.	.982	.93	.786	.734	.904	.92
1 hr.	1.035	.898	---	---	---	---
2 hr.	.907	---	---	---	---	---
5½ hr.	1.056	---	---	---	---	---
8½ hr.	.973	---	.833	.762	1.105	.924
12 hr.	.982	---	---	---	---	---
24 hr.	.973	---	.704	.80	---	.97

Table 8 --- Acute Experiment --- Butanol Extractable Iodine in ug/100 ml

	V.M.	J.W.	P.L.
0 hr.	3.8	---	4.2
1 hr. after TRIPROP	12.0	---	---
2 hr. after TRIPROP	---	---	---
5½ hr. after TRIPROP	19.1	---	16.7
8½ hr. after TRIPROP	12.6	---	---
12 hr. after TRIPROP	11.3	---	---
24 hr. after TRIPROP	11.3	---	---

Table 9 --- Chronic Experiment --- Plasma Total Fatty Acids in meq/L

	Control			Experimental					
	Day 1	Day 2	Day 3	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6
R.M.	8.32	7.26	7.04	8.93	7.82	7.56	7.25	7.28	6.56
E.L.	12.08	12.39	10.51	10.05	8.44	8.48	8.56	7.74	6.88
W.R.	9.96	9.96	11.52	11.13	9.39	10.36	10.83	9.09	9.84
M.W.	8.99	9.95	9.89	10.2	8.29	8.18	8.68	6.56	---

Table 10 --- Chronic Experiment --- Plasma FFA in meq/L

	Control			Experimental					
	Day 1	Day 2	Day 3	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6
R.M.	0.44	0.28	0.27	0.34	0.40	0.59	0.48	0.72	0.50
E.L.	0.80	0.45	0.54	0.305	0.40	0.58	0.64	0.42	0.34
W.R.	0.294	0.408	0.649	1.019	0.447	0.579	0.776	0.779	0.785
M.W.	0.77	0.452	0.336	0.57	0.527	0.696	0.665	0.987	---

* Subjects began taking TRIPROP after collection of all specimens on day 1 of experimental period.

Table 11 --- Chronic Experiment --- Plasma Cholesterol in mg/100 ml

	Control			Experimental					
	Day 1	Day 2	Day 3	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6
R.M.	155.4	140	142.4	145	131	114.5	101.2	93	90.9
E.L.	204	205.8	200	204.1	177.4	169.1	161.8	144.2	125.8
W.R.	162.8	157.8	165.2	167.3	143.8	134.9	145.0	122.0	121.8
M.W.	187	191.5	199.4	205.8	188.8	181.5	168.2	167.5	---

Table 12 --- Chronic Experiment --- Plasma Triglyceride Fatty Acids in meq/L

	Control			Experimental					
	Day 1	Day 2	Day 3	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6
R.M.	0.87	0.42	0.02	2.48	1.97	2.16	2.36	2.57	2.17
E.L.	2.64	2.68	0.82	1.36	1.335	1.09	1.745	1.47	1.51
W.R.	---	1.8	3.73	2.35	1.453	3.264	2.829	1.805	2.934
M.W.	0.21	0.648	0.414	0.65	---	-0.896	0.335	-1.897	---

* Subjects began taking TRIPROP after collection of all specimens on day 1 of experimental period.

Table 13 --- Chronic Experiment --- Plasma Lipid Phosphorus in mg/100 ml.

	Control			Experimental					
	Day 1	Day 2	Day 3	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6
R.M.	7.1	6.8	7.0	5.8	5.2	4.6	4.4	3.9	3.8
E.L.	8.35	9.35	9.35	7.9	6.0	6.3	6.0	6.0	4.6
W.R.	---	8.3	7.0	8.0	8.3	6.9	7.8	7.3	6.6
M.W.	7.8	9.1	9.35	8.8	---	8.45	7.75	7.5	---

Table 14 --- Chronic Experiment --- Respiratory Quotient.

	Control			Experiment					
	Day 1	Day 2	Day 3	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6
R.M.	0.92	0.83	0.82	0.96	0.88	0.99	0.84	0.75	0.78
E.L.	1.08	0.84	0.94	0.90	0.90	0.84	0.86	0.87	0.76
W.R.	0.84	0.87	0.94	0.84	0.96	1.00	0.84	0.80	0.90
M.W.	0.72	0.77	---	0.76	0.70	0.73	0.70	0.75	---

* Subjects began taking TRIPROP after collection of all specimens on day 1 of experimental period

Table 15 --- Chronic Experiment --- Colesterol-Phospholipid Ratio

	Control			Experimental					
	Day 1	Day 2	Day 3	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6
R.M.	0.875	0.824	0.814	1.00	1.007	0.996	0.92	0.954	0.956
E.L.	0.977	0.881	0.856	1.03	1.18	1.07	1.08	0.961	1.094
W.R.	---	0.76	0.944	0.836	0.693	0.782	0.744	0.669	0.739
M.W.	0.959	0.842	0.854	0.934	----	0.859	0.868	0.893	---

Table 16 --- Basal Metabolic Rate --- Comparision in percentage with Standard Values for Comparable Age and Sex

	Control			Experimental					
	Day 1	Day 2	Day 3	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6
R.M.	+2.36	-15.47	-3.45	-0.54	+46.6	-19.79	-4.84	+11.27	---
E.L.	-34.88	-13.56	-3.04	-25.59	-8.62	+2.30	+1.00	+ 9.0	+8.4
W.R.	+14.1	+4.9	+16.4	+3.30	+32.8	+13.03	+35.80	+18.01	+30.7
M.W.	+9.26	-3.26	---	-2.36	+3.29	+13.4	+15.0	+21.6	---

* Subjects began taking TRIPROP after collection of all specimens on day 1 of experimental period.

Table 17--- Chronic Experiment --- Weight in Pounds

	Control			Experimental					
	Day 1	Day 2	Day 3	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6
R.M.	---	140.5	140	139½	138	137	137½	136½	---
E.L.	137½	137 5/8	136½	136 3/4	134 3/8	133¼	132¼	130 3/4	129 3/4
W.R.	---	---	---	174	171½	172½	174	168	167
M.W.	---	---	---	101.3	---	99.06	100.15	98.6	---

Table 18 --- Chronic Experiment --- Pulse Rate in Beats per minute.

	Control			Experimental					
	Day 1	Day 2	Day 3	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6
R.M.	---	62	72	72	82	80	94	---	90
E.L.	---	66	63	69	84	80	84	84	88
W.R.	68	74	61	62	69	62	77	87	87
M.W.	---	---	---	62	80	70	64	76	---

* Subjects began taking TRIPROP after collection of specimens on day 1 of experimental period.

Table 19 --- Chronic Experiment --- Blood Pressure in mm of Hg

	Control			Experimental					
	Day 1	Day 2	Day 3	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6
R.M.	120/82	114/84	120/78	120/78	124/84	110/80	110/78	114/80	112/82
E.L.	---	100/74	110/74	100/74	104/76	102/60	94/66	100/64	100/64
W.R.	130/70	100/50	135/75	110/64	110/72	110/74	120/84	124/74	110/76
M.W.	---	---	---	90/55	100/62	90/50	100/60	90/60	---

Table 20 --- Chronic Experiment --- BEI in ug/100 ml

	Experimental					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
R.M.	---	---	---	---	---	---
E.L.	3.2	---	---	22.2	---	24.4
W.R.	---	---	---	---	---	---
M.W.	4.8	---	28.3	---	19.9	---

* Subjects began taking TRIPROP after collection of all specimens on day 1 of experimental period.

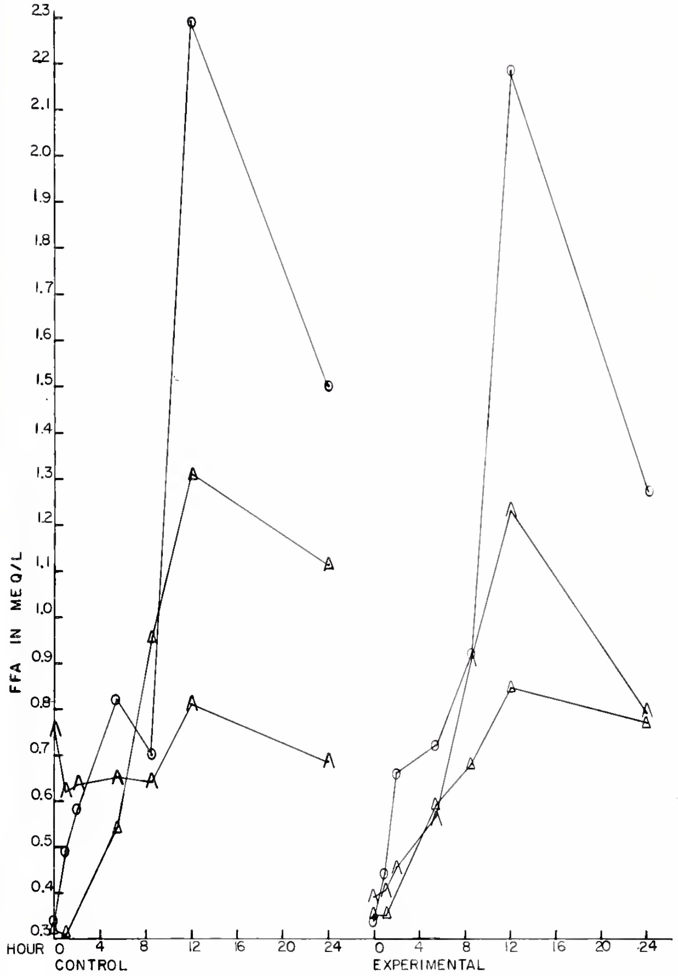


Figure 1 —— Acute Experiment —— Plasma FFA in meq/l

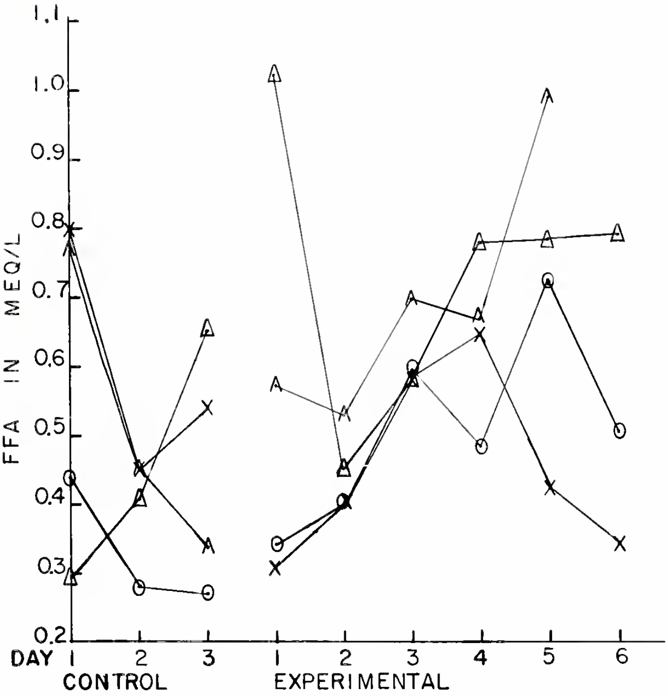


Figure 2 ---- Chronic Experiment ---- Plasma FFA in meq/L

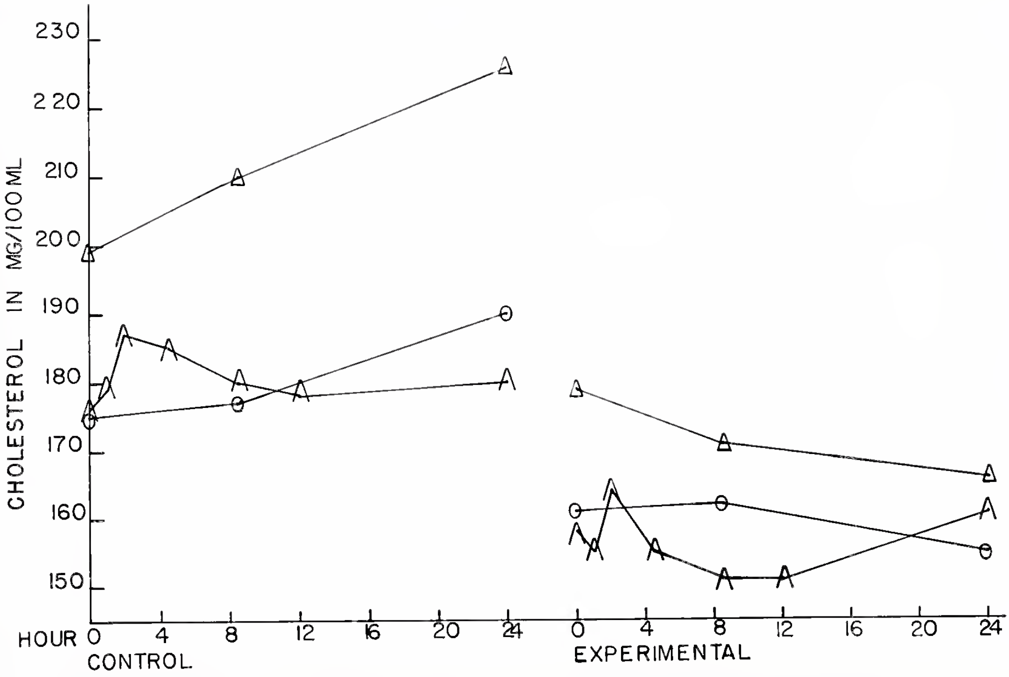


Figure 3 ---- Acute Experiment ---- Plasma Cholesterol in mg/100 ml

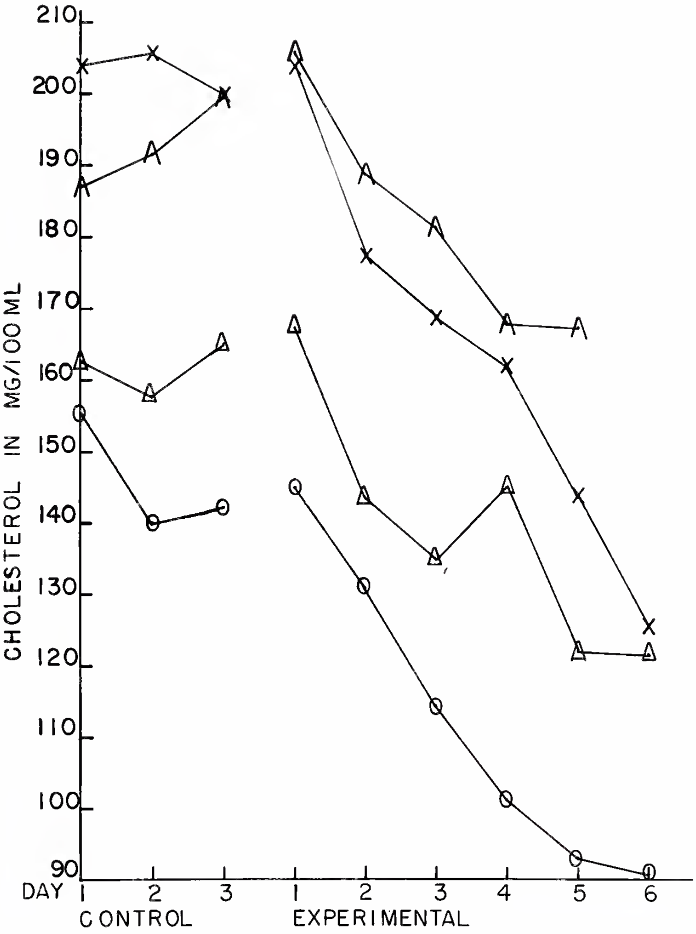


Figure 4 --- Chronic Experiment --- Plasma Cholesterol in mg/100 ml

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